Glycosylation in cancer: mechanisms and clinical implications

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ABSTRACT

Despite recent progress in understanding the cancer genome, there is still a relative delay in understanding the full aspects of the glycome and glycoproteome of cancer. Glycobiology has been instrumental in relevant discoveries in various biological and medical fields, and has contributed to the deciphering of several human diseases. Glycans are involved in fundamental molecular and cell biology processes occurring in cancer, such as cell signalling and communication, tumour cell dissociation and invasion, cell–matrix interactions, tumour angiogenesis, immune modulation and metastasis formation. The roles of glycans in cancer have been highlighted by the fact that alterations in glycosylation regulate the development and progression of cancer, serving as important biomarkers and providing a set of specific targets for therapeutic intervention. This Review discusses the role of glycans in fundamental mechanisms controlling cancer development and progression, and their applications in oncology.

INTRODUCTION

In recent years, glycobiology has gained increased importance in cancer research, given its role in understanding various cancer mechanisms and as it provides a set of targets for diagnostic application and therapeutic strategies1–6. Glycosylation can act as a key regulatory mechanism controlling several physiopathological processes. Defects in glycosylation in humans and their links to disease have shown that the mammalian glycome contains a remarkable amount of biological information7. Glycan diversity arises from differences in monosaccharide composition (for example,

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galactose (Gal) or N-acetylgalactosamine (GalNAc)), in linkage between monosaccharides (for example, between carbons 1 and 3 or carbons 1 and 4), in anomeric state, in branching structures, in other substitutions (such as sulfation state) and in linkage to their aglycone part (protein or lipid)8,9 (FIG. 1). Characterizing the biological functions of each glycan10, as well as those of glycan-binding proteins (including galectins and sialic acid-binding immunoglobulin-type lectins (siglecs)), has been shown to make important contributions to the cancer field1–3,5. Different types of glycoconjugates interfere with key cancer cell processes as well as with the tumour microenvironment, leading to cancer progression. This Review describes how glycans affect and regulate the genesis and progression of cancer. The recent cutting-edge technological developments in glycobiology and their innovative applications in the oncology field are also introduced and discussed.

Glycoconjugates and glycosylation

Glycosylation is defined as the enzymatic process that produces glycosidic linkages of saccharides to other saccharides, proteins or lipids1,11. Glycoconjugates are primarily defined according to the nature of and linkage to their aglycone (non-glycosyl) part (FIG. 1). Glycoproteins carry one or more glycans covalently attached to a polypeptide backbone, usually via nitrogen or oxygen linkages, in which case they are known as N-glycans or O-glycans, respectively8,12,13 (FIG. 2). A common type of protein O-glycosylation is initiated via GalNAc — the first monosaccharide that connects serine or threonine in particular forms of protein O-glycosylation (O-GalNAc) called mucin-type O-glycosylation12,13 — which can be extended into various different structures14. There are other types of O-glycans as well, such as those attached via O-mannose, and the nucleocytoplasmic glycan O-linked β-N-acetylglucosamine (O-GlcNAc)15 (FIGS 1,2). In addition, other forms of glycosylation exist that occur only in specific types of proteins, such as the Notch receptor, and these have been shown to be important in cancer cell biology16 (BOX 1). Moreover, several proteins are linked to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor; these are known as GPI-anchored proteins8 (BOX 2). Other major classes of glycoconjugates include the proteoglycans and glycosphingolipids (FIG. 1). Proteoglycans are glycoconjugates that have one or more glycosaminoglycan (GAG), such as chondroitin sulfate, heparan sulfate and keratan sulfate8. Hyaluronan is a GAG primarily found as a free sugar chain. Glycosphingolipids are molecules composed of a glycan linked to a lipid ceramide. The structural and functional classifications of glycosphingolipids have traditionally been based on their glycan part8. The first sugars linked to ceramide in higher animals are typically β-linked galactose (galactosylceramide) or glucose (glucosylceramide). In vertebrate glycosphingolipids, the glucose moiety is typically substituted with β-linked galactose, creating a lactosylceramide (d-galactosyl-1,4-β-d-glucosylceramide). Glycosphingolipids include a series of neutral 'core' structures and gangliosides, which typically carry one or several sialic acids and have been shown to regulate receptor tyrosine kinase (RTK) signalling17.

Glycosylation alterations in cancer

Changes in glycosylation associated with oncogenic transformation were first described over more than six decades ago18,19. Those observations were further corroborated with the advent of monoclonal antibody technology, which showed that tumour-specific antibodies were directed against carbohydrate epitopes and, in most cases, were oncofetal antigens present on tumour

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glycoproteins and glycosphingolipids20,21. Tumour cells display a wide range of glycosyla- tion alterations compared with their non- transformed counterparts. Protein glycosylation increases molecular hetero geneity as well as the functional diversity within cell populations (FIG. 2). This heterogeneity occurs because aberrant glycan modifications are protein- specific, site- specific (different sites on a given protein can be differentially glycosylated) and cell- specific. The specificities of glycosylation depend on various intrinsic factors of the glycosylation process within a given cell or tissue type. Two principal mechanisms underlying the tumour- associated alterations of carbo- hydrate structures were first postulated by Hakomori and Kannagi, in the so- called incomplete synthesis and neo- synthesis processes22. The incomplete syn- thesis process, occurring more often in early stages of cancer, is a consequence of the impairment of the nor- mal synthesis of complex glycans expressed in normal epithelial cells, which leads to the biosynthesis of trun- cated structures, as seen with sialyl Tn (STn) expression in gastrointestinal and breast cancers23,24. Conversely, neo- synthesis, commonly observed in advanced stages of cancer, refers to the cancer- associated induction of certain genes involved in the expression of carbohydrate determinants, as seen in the de novo expression of cer- tain antigens (such as sialyl Lewis a (SLea) and SLex) in many cancers25. In general, a shift from the normal glycosylation pathway occurs in cancer cells, leading to altered glycans expression owing to one or various factors. First, altered expression of glycans can be attributed to under- or overexpression of glycosyltransferases (owing to dys- regulation at the transcriptional level25–28, dysregulation of chaperone function29,30 and/or altered glycosidase activity31). Second, altered glycan expression can be due to changes in the tertiary conformation of the peptide backbone and that of the nascent glycan chain. Third, the differences can stem from the variability of vari- ous acceptor substrates as well as the availability and abundance of the sugar nucleotide donors and cofac- tors32. Finally, changes in glycan expression can be due to the expression and localization of the relevant glycosyltransferases in the Golgi apparatus33,34. Mislocalization and/or changes in the activity of the glycosyltransferases results in the synthesis of imma- ture core glycan structures35,36. Studies have shown that early acting enzymes synthesizing core O- glycans, such as GalNAc transferases, core 1 GalNAc β1,3- ga lactosyltransferase 1 (C1GalT1) and core 2 β1,6- N- acetylglucosaminyltransferase (C2GnT), are enriched in cis- and medial- Golgi cisternae34,37, whereas late- acting enzymes (such as sialyltransferases) are enriched in trans-Golgi cisternae. In cells, overexpression of α- GalNAc α- 2,6- sialyltransferase I (ST6GalNAc- I; encoded by ST6GALNAC1), the enzyme responsible for STn biosynthesis23,24,38, leads to expression of enzymes in all Golgi cisternae and disrupts glycosylation by pre- maturely adding sialic acid to form the STn antigen36,38. The most- widely occurring cancer- associated changes in glycosylation are sialylation, fucosylation, O- glycan truncation, and N- and O- linked glycan branching2,39,40 (FIGS 2,3).

Sialylation. Sialylation is an important modification in cellular glycosylation, as sialylated carbohydrates have an important role in cellular recognition, cell adhesion and cell signalling. An increase in global sialylation — especially in α2,6- and α2,3- linked sialylation — owing to altered glycosyltransferases expression has been closely associated with cancer41. The lactosaminic chains are frequently terminated with a sialic acid. For example, α2,6- sialylated lactosa- mine (Sia6LacNAc) is the product of β- galactoside α2,6- sialyltransferase I (ST6Gal- I)42, an enzyme with altered expression in various malignancies — includ- ing colon, stomach and ovarian cancer42 — and that has been reported to be a predictive marker of poor progno- sis in colon cancer43. Other major sialylated antigens associated with cancer are SLea and SLex (REF. 2) (FIG. 2). SLea and SLex have been demonstrated to be highly expressed in many malignant cancers, and SLex expression levels have been correlated with poor survival in cancer patients44,45. SLex is the

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well-known ligand for selectins[46], which are vascular cell adhesion molecules that belong to a family of C-type lectins (which require calcium for binding). During inflammation, selectins mediate the initial attachment of leukocytes to the endothelium during the process of leukocyte extravasation[46]. In cancer, SLex interactions with selectins regulate the metastatic cascade by forming emboli of cancer cells and platelets and favouring their arrest on endothelia (FIG. 4), therefore determining the malignant behaviour and development of metastasis[47]. Tumour metastasis has been shown to be attenuated in animal models by the use of specific GAGs, such as heparin, that inhibit P-selectin-mediated interactions of platelets with carcinoma cell-surface ligands[48]. The SLea tetrasaccharide, which is detected by the serological assay CA19-9, is a cancer-associated marker widely used in the clinical practice. The CA19-9 assay has been mostly applied in patients with an established diagnosis of pancreatic, colorectal, gastric or biliary cancer and used to monitor clinical response to therapy[3,49]. In addition, elevated preoperative concentrations of CA19-9 have been shown to be associated with poor prognosis in colon and gastric carcinoma[50]. Increased sialylation in cancer also includes the expression of polysialic acid, which is associated with several types of cancers and is frequently expressed in high-grade tumours[51,52]. Polysialic acid can often be present in neural cell adhesion molecule 1 (NCAM1), and this is associated with aggressiveness and poor clinical outcome in cancers, including lung cancer, neuroblastoma and gliomas[51,52]. Gangliosides are also overexpressed in tumours such as melanoma, neuroblastoma and breast cancer, in which they mediate cell proliferation, tumour growth and cancer cell migration[17,53].

Box 1 | **The unique type of Notch glycosylation**

Notch signalling is essential for cell fate, and dysregulation of the pathway leads to various human diseases, including cancer[96]. Glycosylation of the Notch extracellular domain has been shown to regulate Notch activity[96]. The Notch ligands (the Delta, Serrate and LAG-2 family of proteins) bind to the extracellular domain of Notch receptor, triggering its activation by inducing a conformational change that exposes cleavage sites in Notch. Cleavage at these sites results in liberation of the Notch intracellular domain, which translocates to the nucleus and controls the transcriptional activation of the transcription factor recombining binding protein suppressor of hairless (RBP-Jκ). The Notch extracellular domain is modified with different types of carbohydrates, including Asp-linked *N*-glycans and several *O*-glycans, such as *O*-fucose[208]. *O*-fucose monosaccharides are elongated to a *N*-acetylglucosamine β1-3fucose (GlcNAcβ1-3Fuc) disaccharide by the action of the Fringe *N*-acetylglucosaminyltransferase in *Drosophila melanogaster* and by the Fringe homologues in vertebrates[96,209]. The disaccharide can be further elongated to the tetrasaccharide Neu5Acα2-3/6Galβ1-4GlcNAcβ1-3Fuc by the sequential action of several glycosyltransferases in mammals[96].

Fringe was demonstrated to be a modulator of Notch activity[209]. Three Fringe homologues exist in mammals: lunatic fringe, manic fringe and radical fringe[210]. Notch regulation by glycosylation, such as the addition of GlcNAc by Fringe, was shown to interfere with Notch–ligand interactions, promoting Notch–Delta binding and reducing Notch–Serrate binding[96,209].

Several studies have reviewed the mechanisms of glycosylation in the regulation of this important receptor in cancer[96]. Glycosylation-dependent modulation of Notch signalling controls development, maintains tumour cell 'stemness' and mediates cancer metastasis[96].

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Fucosylation. Fucosylation has been also associated with cancer. Fucosylated glycans are synthesized by a range of fucosyltransferases (Fuc-Ts; Fuc-TI–Fuc-TXI (encoded by FUT1–FUT11, where FUT3 is also known as the Lewis gene, Le)), with fucosylation existing as a non-extendable modification and being generally sub-divided into terminal fucosylation (giving rise to specific Lewis blood-group antigens, such as Lex and Ley and Lea and Leb) and core fucosylation54. The terminal steps of the biosynthesis of SLe antigens include the α1,3- or α1,4-fucosylation of a previously α2,3-sialylated type 1 (SLea) or type 2 (SLex) chains54,55 (FIG. 2). The enhanced expression of SLex in adult T cell leukaemia cells has been shown to be dependent on Fuc-TVII activity. The aetiologic agent of this leukaemia, the human T-lymphotropic virus 1 (HTLV-1) retrovirus, encodes a transcriptional activator protein, TAX, which regulates the FUT7 gene encoding Fuc-TVII, the limiting enzyme controlling SLex synthesis in leukocytes56. In breast tumours, the expression of SLex seems to be regulated mainly by Fuc-TVI (encoded by FUT6)57. However, the biosynthesis of SLe antigens in gastrointestinal cancer may depend on the coordinated expression of several glycosyltransferases. The expression of both SLex and SLea antigens expressed by glycolipids in colon cancer tissues has been related to the activation of a β1,3GlcNAc transferase; this enzyme synthesizes a sugar chain that is a precursor for both type 1 and 2 Lewis structures58. A similar mechanism was observed in gastritis induced by Helicobacter pylori59,60, a bacterium that expresses adhesins that recognize glycan receptors expressed by the gastric epithelium subsequently causing gastric ulcers and, potentially, gastric carcinogenesis5 (BOX 3). Fuc-TVI has also been reported as a major enzyme modulating the SLex biosynthesis in colorectal cancer (CRC)61. Core fucosylation consists in the addition of α1,6-fucose to the innermost GlcNAc residue of N-glycans through the action of Fuc-TVIII (encoded by FUT8) (FIG. 2). Overexpression of FUT8 and core fucosylation is an important feature in several cancers, such as lung cancer and breast cancer62,63. This increased core fucosylation is reflected in the serum levels during the process of hepatocarcinogenesis64. Interestingly, core fucosylation of α-fetoprotein is an approved biomarker for the early detection of hepatocellular carcinoma (HCC), distinguishing it from chronic hepatitis and liver cirrhosis65. In breast cancer, increased core fucosylation of epidermal growth factor receptor (EGFR) was associated with increased dimerization and phosphorylation, which resulted in increased EGFR-mediated signalling associated with tumour cell growth and malignancy62,66.

Branching and bisecting GlcNAc N-glycans. During malignant transformation, a frequently occurring glycosylation change in cancer cells is the increased expression of complex β1,6-branched N-linked glycans2,67 (FIGS 2,3). Increased GlcNAc-branching N-glycan expression is due to increased activity of GnT-V, which is encoded by the mannoside acetylglucosaminyltransferase 5 (MGAT5) gene. MGAT5 expression is regulated by the RAS–RAF–MAPK signalling pathway, which is activated in cancer67. Branched N-glycans are further modified by β1,4-GalTs and elongated with poly-N-acetyllactosamine (repeats of Galβ1,4GlcNAcβ1,3) by β1,3-GnTs, and further capped with sialic acid and fucose. This poly-N-acetyllactosamine structure is a ligand for galectins, a family of conserved carbohydrate-binding proteins, which form galectin–glycan structures termed 'lattices' (REF. 68). Galectins have important roles in cancer, contributing to neoplastic transformation, tumour cell survival, angiogenesis and tumour metastasis69. Overexpression of MGAT5 in an immortalized lung epithelial cell line resulted in loss of contact inhibition, increased cell motility and tumour formation in athymic mice70, as well as in enhanced invasion and metastasis in mouse mammary carcinoma cells71. Moreover, early events in breast carcinoma formation in a Her2-transgenic mouse mammary tumour model were found to be regulated by GnT-V72. In addition, downregulation of GnT-V in mouse mammary cancer cell

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lines resulted in a significant suppression of tumour growth and metastasis71. Breast cancer progression and metastasis induced by a viral oncogene in transgenic mice is markedly suppressed in Mgat5-deficient background73. Moreover, GnT-V-mediated glycosylation regulates the colon cancer stem cell compartment and tumour progression through WNT signalling74. In contrast to the function of GnT-V, GnT-III (encoded by MGAT3) catalyses the addition of bisecting GlcNAc N-glycans in a β1,4-linkage, suppressing additional processing and elongation of N-glycans such as the β1,6-branching structures. GnT-III counteracts the role of GnT-V in cancer, being involved in the suppression of cancer metastasis75. MGAT3 transfection into mouse melanoma B16 cells with high metastatic potential resulted in a significant reduction of β1,6GlcNAc branching (owing to GnT-III and GnT-V enzymatic competition), leading to a significant suppression of lung metastasis in mice. GnT-III suppresses tumour metastasis through the regulation of key glycoproteins, such as EGFR, integrins and cadherins66,76, as described below.

Truncated O-glycans. Another common feature of tumours is the overexpression of truncated O-glycans (FIGS 2,3). The GalNAc-type O-glycans, also called mucin-type O-glycans, are frequently found in most transmembrane and secreted glycoproteins. During malignancy, aberrant glycosylation also occurs in glycoproteins that display abnormal expression of shortened or truncated glycans, such as the disaccharide Thomsen–Friedenreich antigen (T antigen, also known as core 1) and the monosaccharide GalNAc (also known as Tn) and their sialylated forms (ST and STn (Neu5Acα2-6GalNAcα-O-R), respectively), which result from the incomplete synthesis of O-glycans77. Altered expression of polypeptide GalNAc transferases (ppGalNAcTs) — the enzymes initiating the mucin-type O-glycosylation12,13 — is often observed in cancer78,79. The ppGalNAcTs control the sites and density of O-glycan occupancy12,13, and changes in their expression lead to alterations in O-glycosylation80. In addition, enzymes competing for the same substrate can also induce expression of truncated glycans and exposure of protein epitopes that would otherwise be hidden in the normally glycosylated protein. The relative enzymatic activities of C2GnT and α2,3-sialyltransferase I (ST3Gal-I) have been shown to determine the O-glycan structure in cancer cells81. These relative activities underlie the aberrant expression of a tumour-associated epitopes on glycoproteins, such as mucins in breast81 and gastric82 cancers. STn is rarely expressed in normal healthy tissues but can be detected in most carcinomas, such as those from the pancreas83,84, stomach23,85,86, colorectum23,87, breast38, bladder88 and ovary89, correlating with decreased cancer cell adhesion, increased tumour growth, increased tumour cell migration, invasion and poor prognosis. The abnormal synthesis of STn in cancer occurs owing to the overexpression of ST6GalNAc-I. Mutations in T-synthase C1GalT1-specific chaperone 1 (C1GALT1C1) — which blocks further O-glycan elongation and shifts the pathway towards generation of Tn — can also lead to STn expression through the action of ST6GalNAc-I90,91 (FIG. 2).

Therefore, STn has been proposed as an important prognostic marker and a target for the design of anticancer vaccines92,93.

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Box 2 | **GPI-anchored proteins and disease**

Glycosylphosphatidylinositol (GPI)-anchored proteins are formed by a glycan bridge between phosphatidylinositol and a phosphoethanolamine, which is then linked to the carboxy-terminal amino acid of a protein. This structure typically constitutes the only anchor to the lipid bilayer membrane for some proteins8. Mutation in the GPI phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A (*PIGA*) gene leads to defects in the synthesis of the GPI anchor, resulting in deficiency of all GPI-bound proteins. Haematopoietic stem cells that are defective in GPI anchor assembly owing to a mutation in the *PIGA* gene preferentially expand in the bone marrow and give rise to defective peripheral blood elements that are deficient in GPI-anchored protein expression. Mutation in the X-linked *PIGA* gene causes paroxysmal nocturnal haemoglobinuria, a disease characterized by haemolytic anaemia, thrombosis and impaired bone marrow function, with an increased risk of developing leukaemia211.

Glycosylation in the cancer cell

Glycans have been found to participate in numerous fun- damental biological processes involved in cancer, such as inflammation (BOX 3), immune surveillance, cell–cell adhesion76,94,95, cell–matrix interaction76, inter- and intracellular signalling96–99, and cellular metabolism100,101 (FIG. 4). Furthermore, glycans alter protein conformation and structure, thereby modulating the functional activity of the protein102. Unravelling the biological significance of glycan-based interactions in cancer can contribute to the deciphering of molecular mechanisms underlying the biology of cancer.

Glycosylation in tumour cell–cell adhesion. The devel- opment of malignant tumours is in part characterized by the ability of a tumour cell to overcome cell–cell adhesion and to invade surrounding tissue. Epithelial cadherin (E-cadherin) is a transmembrane glycopro- tein103 and a major epithelial cell–cell adhesion mole- cule in cancer104. Glycans can have a profound effect on tumour cell–cell adhesion by directly interfering with E-cadherin functions. GnT-V overexpression in gastric cancer cells induces E-cadherin cellular mislocalization from the cell membrane into the cytoplasm and its functional impairment94,95. The addition of GnT-V-mediated β1,6GlcNAc-branched N-glycans to E-cadherin leads to incorrectly assembled and non-functional adherens junctions, which compromise cell–cell adhesion94,95,105 and downstream signalling pathways106, contributing to tumour invasiveness and metastases107. Preventing this aberrant glycosylation in a specific Asp site improves E-cadherin functions in cancer108. Interestingly, patients with gastric carcinoma displaying loss of E-cadherin function (not explained at the genetic or structural level) exhibit an increase in β1,6GlcNAc-branched N-glycans on E-cadherin5,94. Conversely, GnT-III-mediated bisecting GlcNAc N-glycans counteract GnT-V activity through E-cadherin regulation75,94. This E-cadherin glycan modification was associated with a delayed turnover rate at cell membrane94,109, inhibition of endocytosis94, decreased phosphorylation of β-catenin that remained in complex with E-cadherin110, and increased stability of adherens junctions, promoting tumour suppression5,94,95. Moreover, expression of GnT-III is also associated with suppression of epithelial-to-mesenchymal transition28,111. Therefore, a mutual regulatory mechanism between E-cadherin-mediated cell–cell adhesion and its glycosylation exists in cancer, which is controlled by the competitive action of GnT-III and

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GnT-V, and can culminate in either tumour suppression or tumour metastasis, respectively[5,112] (FIG. 4). Cancer cells produce increased levels of sialylated glycans, leading to the high expression of tumour-associated antigens[2,113]. Increased expression of sialylated antigens promotes cell detachment from the tumour mass through electrostatic repulsion of negative charges, which physically inhibits and disrupts cell–cell adhesion[114,115]. Transfection of breast cancer cells with ST6Gal-I results in increased cell migration and decreased cell–cell adhesion in vitro[116] (FIG. 4). Furthermore, sialylated glycans (such as SLex) can promote the adhesion of tumour cells to vascular endothelial cells through their interaction with selectins, such as E-selectin, mediating the initial steps of the formation of cancer metastases[2] (FIG. 4). In addition, de novo expression of STn in gastric carcinoma cells modulates the malignant phenotype, inducing more-aggressive cell behaviour, with decreased cell–cell aggregation and increased matrix interaction, migration and invasion[85]. RNA interference-mediated gene silencing of ST6GALNAC1 suppresses the metastatic potential of gastric cancer cells owing to a reduction in expression of insulin growth factor I (IGF-I) and reduced activation of signal transducer and activator of transcription, STAT5B [117]. Furthermore, somatic mutations and hypermethylation of C1GALT1C1 have shown that loss of C1GALT1C1 function leads to STn expression, preventing cell–cell interactions and contact inhibition of cell growth in cancer cells[84]. Clinically, increased sialylation is often associated with invasiveness and poor prognosis of cancer patients[44,47].

Glycosylation in cell–matrix interaction and signalling. The extracellular matrix (ECM) is composed of a dynamic and complex array of glycoproteins, collagens, GAGs and proteoglycans. It provides mechanical and structural support, as well as spatial context, for signalling events, with direct implications in tumour development, maintenance of stem cell niches and cancer progression[118]. Heparan sulfate proteoglycans (HSPGs) are present on the cell surface and in the ECM and can modulate cell growth and differentiation, controlling embryogenesis, angiogenesis and homeostasis. HSPGs contain one or more covalently attached heparan sulfate GAG chains[119]. There are different groups of HSPGs classified according to their location: membrane HSPGs, such as syndecans and the GPI-anchored proteoglycans, the glypicans; the ECM HSPGs, such as agrin, perlecan and type XVIII collagen; and the secretory-vesicle HSPG, serglycin[119]. HSPGs can bind cytokines, chemokines and growth factors, protecting them against proteolysis; in addition, HSPGs can act as co-receptors for various growth factors for tyrosine kinase receptors, lowering their activation thresholds or changing the duration of their signalling reactions[119] (FIG. 4). Overexpression of proteoglycans occurs in several cancers in which the heparan sulfate chains covalently bound to the proteoglycans can modulate the activation of protein receptors, such as HER2, EGFR, MET (also known as hepatocyte growth factor receptor (HGFR)) and transforming growth factor-β (TGFβ)[120]. Heparan sulfate chains regulate the interactions[121], and increase the solubility, of various signalling molecules[122], therefore increasing their access to receptors and facilitating signal transduction. For instance, heparan sulfate chains can release HGF, inducing cell growth and motility through interaction with MET[121], which is frequently activated in cancer cells[99] (FIG. 4). Heparan sulfate chains can also release vascular endothelial growth factor A (VEGFA), a regulator of angiogenesis that stimulates growth, motility, and tubulogenesis in vascular endothelial cells through interactions with VEGF receptor 1 (VEGFR1) and VEGFR2 (REF. 121). Another important membrane receptor involved in matrix-dependent cell motility and migration is CD44, which is the main receptor for hyaluronic acid. CD44 is a multifunctional cell surface molecule involved in cancer cell proliferation, differentiation, migration and signalling[123]. CD44 splicing variants have been associated with tumour development and progression[124]. The role of CD44 glycosylation in matrix-dependent cell adhesion, motility and

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migration is far from being elucidated. Nevertheless, evidence has shown that changes in glycosylation of CD44 can markedly influence hyaluronic acid ligand recognition and binding, modifying cancer cell signalling125. Treatments of CD44 with inhibitors of glycosylation and de-glycosylating enzymes signifi- cantly change the binding to hyaluronic acid, modulating CD44-dependent signalling and function126. Moreover, glycosylation modification of CD44 induced by transfection of α1,2–Fuc-T enhanced cell motility and tumori- genicity in rat carcinoma cells127. Additionally, GAG forms of CD44 containing chondroitin and heparin sul- fate chains modulate the binding of tumour cells to fibro- nectin128. Proteoglycans are also involved in the biogenesis and recognition of exosomes, which are secreted vesicles of endosomal origin involved in signalling processes129. Syndecans control the interaction with key acces- sory components of the endosomal-sorting complexes required for transport machinery. In addition, hepara- nase modulates syndecan-controlled pathways, foster- ing endosomal membrane budding and the biogenesis of exosomes by trimming the heparan sulfate chains on syndecans and controlling the selection of specific cargo to exosomes129. Hyaluronidases also have many roles in cancer metastasis by participating in the degradation of the ECM surrounding the tumour, enabling cancer cells to disseminate from the primary tumour and allowing invasion by degradation of the basement membrane and by clearing the ECM of the secondary site130. Recent studies demonstrated that expression of bulky glycoproteins in the cancer cell glycocalyx facilitates integrin clustering by funnelling active integrins into adhesions and by applying tension to matrix-bound integrins, independently of actomyosin contractility131. Expression of large tumour-associated glycoproteins in non-transformed cells facilitates integrin-dependent growth factor signalling to support cell survival, further confirming that alterations of glycoprotein expres- sion in the cancer cell glycocalyx could foster invasion and metastasis by mechanically enhancing cell-surface receptor function131. Cell–ECM interactions play essential parts during the acquisition of migration and invasive behaviour of tumour cells132. Integrins are carriers of N-glycans and are important receptors for signals in the ECM and connect many biological functions, such as cell proliferation, protection against apoptosis and malignant transformation131. Integrin expression is upregulated in migratory cells associated with tumour metastases133. N-glycans on α5β1 integrin, a receptor for fibronectin (encoded by FN1), are required for αβ-heterodimer formation and for proper integrin–matrix inter action76. Changes in N-glycans in cancer can regulate integrins functions. Transformation of NIH3T3 cells with an oncogenic RAS gene resulted in enhancement of cell spreading on fibronectin due to increased modifica- tion of α5β1 integrins with β1,6GlcNAc-branching N-glycans134 through the upregulation of the

RAS–RAF–MAPK signalling pathway and subsequent activation of MGAT5 transcription. Similarly, over- expression of human fibrosarcoma cells with GnT-V resulted in an increased cell migration towards fibro- nectin and invasion through the Matrigel due to an increase in β1,6-branching N-glycans on α5β1 integrin135. Moreover, the characterization of carbohydrate moieties of α3β1 integrin, the receptor for laminin-5 showed that β1,6GlcNAc-branched structures were highly expressed in metastatic human melanoma cells136. Changes in N-linked β1,6-branching occurring dur- ing oncogenesis alter cell–matrix adhesion and migration by inhibiting the clustering of integrins and subsequent signal transduction pathways136. In contrast to the over- expression of GnT-V, the overexpression of GnT-III resulted in an inhibition of α5β1 integrin-mediated cell spreading and migration, and the phosphorylation of focal adhesion kinase (FAK). The affinity of the binding of α5β1 integrin to fibronectin was greatly reduced as a result of the introduction of a bisecting GlcNAc N-glycans on the α5 subunit137. Similarly, in MKN45 gas- tric cancer cells, the overexpression of GnT-III suppresses α3β1 integrin-mediated cell migration on laminin-5,

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counteracting the GnT-V activity138. Overall, GnT-III is described to suppress cancer metastases by at least two major mechanisms: an enhancement in cell–cell adhesion and a downregulation of cell–ECM adhesion139. Furthermore, an increased terminal α2,6-sialylation of integrins N-glycans can control cancer cell migratory and metastatic potential, interfering with the ligand-binding properties of integrins101,140. Analysis of cancer cells that overexpress ST6Gal1 consistently indicates altered adhesion of cells to ECM substrates, such as colla gen, fibronectin and laminin in colon cancer141 and breast cancer cell lines116. Additionally, altered N-glycosylation of integrins can have an impact on their cis-interaction with membrane-associated receptors, including EGFR142 and the tetra-spanin family of proteins, as well as gangliosides in the microdomain. Glycosylation of α3β1 integrin was dem-onstrated to regulate its association with the tetra spanin CD151, modulating cell spreading and motility143. Therefore, changes in the N-glycosylation profile of integ-rins modulate tumour cell motility and migration through interference with the supramolecular complex formation (tumour cell focal adhesions) on the cell surface. In the formation of these focal adhesions, integrins interact with HSPG on the surface of tumour cells144. Syndecan-4 is frequently upregulated in a range of cancers145; it binds to fibronectin and laminin-5 enhancing the function of β1 integrin during cell spreading146. Similarly, syndecan-1 was described to functionally couple with αvβ3 integrin in breast cancer cells, resulting in increased αvβ3-dependent cell spreading and migration147.

Box 3 | **Glycosylation at the interface of inflammation-induced cancer**

During inflammation, a considerable number of glycosylation changes occur, and some of these have been associated with the carcinogenesis process. *Helicobacter pylori*, a Gram-negative bacterium specialized in the colonization of the human stomach, can cause gastric ulcers, and persistent infection may cause chronic atrophic gastritis with the development of intestinal metaplasia, dysplasia and gastric carcinoma5. The adhesion of *H. pylori* to the gastric mucosa is mediated by different bacterial adhesins that recognize glycans expressed by the gastric mucosa. The antigen-binding adhesin BabA binds to fucosylated antigens normally expressed by secretor individuals212, and the sialic acid-binding adhesin SabA recognizes sialylated Lewis glycans (sialyl Lewis a (SLea) and SLex) expressed in gastritis213. Inflammation-induced glycosylation alterations, such as the aberrant overexpression of SLex, occur because of changes in glycosyltransferases expression59,60,214. Changes in glycosylation have also been studied in acute-phase proteins, such as α1 antitrypsin, as potential biomarkers in cancer and in acute and chronic inflammatory conditions215. Furthermore, glycosylation alterations have been shown to correlate with disease severity in certain inflammatory conditions, such as in inflammatory bowel disease216. In addition, glycosylation alterations have been reported in circulating proteins produced by the liver in patients with inflammatory diseases, such as gastritis203 and pancreatitis215.

Several studies have shown that the sialic acid *N*-glycolylneuraminic acid (Neu5Gc) is enriched in red meat, an epidemiological risk factor for cancer development217. Humans cannot synthesize Neu5Gc because the human gene cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase (*CMAH*), which encodes the enzyme responsible for the synthesis of CMP-Neu5Gc from CMP-*N*-acetylneuraminic (CMP-Neu5Ac) acid is irreversibly mutated. The active form of CMAH is found in apes218, and the mutated CMAH form is estimated to have originated 2–3 million years ago, prior to the emergence of the genus *Homo*218. Neu5Gc has been shown to be bioavailable, undergoing metabolic incorporation into human tissues. Human-like *Neu5Gc*-deficient mice have been shown to develop inflammatory conditions when fed with Neu5Gc and challenged with

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Neu5Gc-specific antibodies. Such mice developed hepatocellular carcinomas217. These studies demonstrate the potential role in cancer development of the sialic acid Neu5Gc and provide an explanation for the epidemiological association between red meat consumption, inflammation and cancer risk.

Glycosylation in cancer metabolism and signalling. A key feature of cancer cell metabolism is a shift from oxidative phosphorylation to aerobic glycolysis (the Warburg effect)148, which is characterized by high rates of glucose uptake to cope with the increased energetic and biosynthetic needs to generate a tumour. Additionally, to help meet increased biosynthetic demands, cancer cells also upregulate glutamine uptake. The abundance of glucose in the cytoplasm of cancer cells not only contributes to increased glycolysis but also increases flux into the metabolic branch pathways, such as the hexo samine biosynthetic pathway (HBP). Approximately 3–5% of the total glucose entering a cell is shunted through this pathway149. Therefore, increased glucose and glutamine uptake by cancer cells probably drives increased HBP flux. The end-product of HBP is uridine diphosphate (UDP)-GlcNAc, which is a critical metabolite that is subsequently used for O-GlcNAcylation as well as for O- and N-glycosylation150. Given O-GlcNAcylation responsiveness to the glucose flux, O-GlcNAc can act as a 'nutritional sensor' (REF. 151). Increased levels of O-GlcNAc transferase (OGT) have been found in breast cancer, and knockdown of OGT in vitro reduces cancer hyper-O-GlcNAcylation and inhibits tumour growth, invasion and metastasis, further indicating that elevated O-GlcNAc contributes to cancer progression152–154. Moreover, O-GlcNAc modulates key protein functions by regulating protein phosphorylation, altering protein degradation, controlling protein localization and mediating transcription155. O-GlcNAc modifications have been implicated in key molecular events occurring in cancer, such as tumour cell proliferation (by regulating the activities of transcription factor forkhead box protein M1(FoxM1) and cyclin D1, which are both involved in cell cycle progression154), cancer cell survival and angiogenesis (through the effect of hyper-O-GlcNAcylation (via activation of nuclear factor κB-mediated signalling153) and upregulation of VEGFA and matrix metalloproteinases (MMPs)156, respectively), and cancer cell invasion and metastasis (through O-GlcNAc regulation of E-cadherin trafficking and function)157. Many oncogene and tumour-suppressor gene products were shown to be modified by O-GlcNAc158. MYC undergoes O-GlcNAcylation at Thr58, which is also a phosphorylation site. In fact, O-GlcNAcylation has extensive crosstalk with phosphorylation and serves as a nutrient sensor to modulate signalling, transcription and cytoskeletal functions158. Altered phosphorylation events affect GlcNAcylation levels and vice versa. Increased MYC O-GlcNAcylation competes with phosphorylation, stabilizing MYC and thus contributing to oncogenesis159. This type of interplay also occurs with the p53 tumour-suppressor protein160. Similarly to O-GlcNAcylation, N-glycan branching is nutrient sensitive, with functional consequences for the cancer cell. The degree of N-glycan branching modulates the activity and/or signalling and surface retention of many cell surface proteins, including growth factor receptors97. Cell surface glycoprotein receptors have different number of N-glycan sites. The number of N-glycans is defined by the protein sequence of each glycoprotein, and the types of N-glycan structures are determined by the Golgi N-glycan-processing pathway and metabolite supply to sugar–nucleotide pools161. Receptors that stimulate cell proliferation, growth and oncogenesis (such as EGFR, IGF receptor (IGFR), fibroblast growth factor (FGFR) and platelet-derived growth factor (PDGFR)) have more N-glycan sites (8–16 Asn-X-Ser/Thr sites, in which X is any amino acid) per 100 amino acids, and longer extracellular domains. Conversely, growth-arrest receptors involved in organogenesis and differentiation (such as TGFβ receptor 1 (TGFβR1) and TGFβR2) have few N-glycan sites161. Lau et al. proposed a mechanism for metabolic regulation of cellular

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transition between cell proliferation and arrest and/or differentiation that arises from the cooperation of complex N-glycan number and the degree of branching structures161. Changes in metabolic flux through the HBP affect the stability and retention of receptors at the cell surface by modulating the interaction of branched N-glycans with galectin-3 (REFS 162,163). The galectin-3 lattice restricts receptor endocytosis, enhancing the signalling68,161. Hence, the more N-glycan sites, the more β1,6-branching structures are added, which crosslink with galectins, precluding endocytosis and thereby increasing signalling161,162. Mammary carcinoma cells derived from polyomavirus middle T (PyMT) Mgat5−/− transgenic mice are less responsive to IGF, EGF, PDGF, FGF and TGFβ compared with Mgat5+/+ tumour cells, showing reduced galectin-3 binding and internalization of receptors from the cell surface to endosomes164. Similarly, human cancer cells with targeted silencing of the MGAT5 gene also exhibit reduced EGFR signalling165. Sensitivity to EGF and TGFβ cytokines was rescued by hexosamine supplementation with UDP-GlcNAc or by GnT-V expression, implying that remodelling of N-glycans in tumour cells is sensitive to metabolism161. Accordingly, the decrease of galectin lattice interactions induced by the addition of bisecting GlcNAc N-glycans counterbalances the highly branched N-glycosylation of EGFR and PDGFR, restraining its downstream signalling and in this way retarding mammary tumour progression166. GnT-III overexpression reduces the ability of EGF to bind to its receptor, blocking EGFR-mediated ERK phosphorylation and increasing EGFR endocytosis167. Increasing intracellular metabolic flux with UDP-GlcNAc promotes a hyperbolic activation profile for high-n receptors (receptors with a high number of N-glycan sites (growth receptors)) and a sigmoid or switch-like profile for low-n receptors (receptors with a reduced number of N-glycan sites (arrest receptors)), thereby regulating the transition between cell growth and differentiation161. Overall, the nutrient flux that regulates complex N-glycan biosynthesis coordinates the cellular response of tumour cells determining growth, invasion and drug sensitivity100. Interestingly, the presence of branching N-glycans on VEGFR2 interacting with galectin-1 underlies an aberrant and compensatory angiogenesis mechanism associated with tumour growth in tumours resistant to anti-VEGF treatment69. Gangliosides have been described as important modulators of signal transduction. Ectopic expression or inhibition of specific glycosyltransferases modifying gangliosides regulates RTK signalling. Within glycolipid-enriched microdomains, RTKs can be modulated by glycans, resulting in inhibition of ligand-induced dimerization and autophosphorylation or in activation of receptor signalling without ligand binding. The RTK modulation depends on the glycan structure; monosialogangliosides (such as GM_3 and GM_1) are considered negative regulators of RTKs, whereas disialo-gangliosides (such as GD_2, GD_3, GD1a and GD1b) are considered activators of RTKs17. Furthermore, physio-pathological changes in cell membrane ganglioside com-position have been shown to result in different cellular responses168. Several growth factor receptors, including EGFR, FGFR, PDGF, MET and IGFR, are regulated by gangliosides17,53,169. RTKs are located in glycolipid-enriched microdomains, and changes in gangliosides modify the molecular composition and the structure of glycolipid-enriched microdomains, leading to modifi-cations in the location and organization of RTKs in the cellular membrane and altered activation53,169. Further regulation of specific ganglioside GD_3 due to formation of 9-O-acetyl GD_3 that renders GD_3 unable to induce apoptosis has been shown in gliomas170.

Glycans in tumour immune surveillance

Glycans regulate various aspects of the immune response interfering with tumour editing. Such regulation is mediated by various lectins — such as galectins, C-type lectins and siglecs — that bind glycans and regulate immune processes such as those relevant for pathogen recognition, thereby

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defining the course of adaptive immune responses171,172. Cancer immune surveillance is an important host protection process thought to inhibit carcino- genesis and maintain cellular homeostasis. Transformed cells can be eliminated by immune effector cells, resulting in immune selection of tumour cell variants with decreased immunogenicity and resistance to immune effector cells. Glycan- specific natural and induced antibodies (such as those against GM2, globo H and Ley) can mediate tumour cell killing and tissue destruction by complement- dependent cytotoxicity173. In addition, aberrant O- glycosylation on the surface of cancer cells can induce antibody- dependent cellular cytotoxicity (ADCC)174 and may interact with dendritic cell- specific intercellular adhesion molecule- 3 grabbing non- integrin 1 (DC- SIGN; also known as CD209)175 and macrophage galactose- type C- type lectin176 expressed on denditric cells. Galectins can also modulate the immune and inflammatory responses and might have a key role helping tumours to escape immune surveillance, therefore having diagnostic and prognostic applications171,177–179. Targeting altered glycosylation as an immunothera- peutic strategy — for example, with anticancer vaccines that target tumour- associated carbohydrate antigens180 — provides an appealing option for cancer treatment92. Examples include vaccines targeting the mucin- related Tn, STn, and T antigens for suppression of breast cancer, the gangliosides GM2 and GD3 for treatment of mela- noma, and the glycosphingolipid globo- H for prostate cancer treatment181. Some of these anticancer vaccines can be designed to incorporate only those elements required for a desired immune response182–184. Antibodies targeting GD2 disialo- ganglioside have been tested in numerous clinical trials in neuroblastoma with impressive antitumour effects and survival outcomes185. Passive immunotherapy using antibodies directed to glycoform- specific targets expressed in tumour cells can be effective at inducing ADCC174. Other studies have shown that ADCC is a key mechanism by which some currently used therapeutic antibodies mediate their antitumour effects. Variations of glycosylation on the heavy chain of the therapeutic antibodies can increase the affinity between the antibody and Fcγ receptor, resulting in increased ADCC186.

Glycans in cancer diagnosis and treatment

New approaches for cancer early diagnosis, risk prediction and treatment are urgently needed, and glycans can be a source for the development of new non- invasive biomarkers. Some of the most- common clinically utilized sero- logical biomarkers for cancer diagnosis and monitoring of malignant progression, as well as prognostic biomarkers of disease recurrence, are glycoproteins3,49. These include prominent biomarkers that are widely used in patients with prostate cancer (prostate- specific anti- gen (PSA))187, ovarian cancer (carcinoma antigen 125 (CA125; also known as mucin- 16 (MUC16)))188, colon cancer (SLea, CA19- 9, 3,49 and carcinoembryonic antigen (CEA)189), breast cancer (aberrantly glycosylated MUC1 (also known as CA15- 3))190,191 gastric cancer (SLea, CA19- 9)3,49 and pancreatic cancer (SLea,, CA19- 9)192 (TABLE 1). Although all of these serological biomarkers have been shown to have an aberrant glyco sylation in cancer193–195, they have limited application owing to their relative low specificity, precluding their use for screening strategies and diagnostic potential. The reduced specificity and sensitivity of these assays for early detection of cancer has driven a search for novel biomarkers based on the detection and measurement of specific glyco- forms of a certain protein that could contribute to the establishment of a biomarker with higher specificity for early detection of cancer or for diagnosis at a precancerous stage. A story of success is that of α- fetoprotein (AFP), a glyco- biomarker used for the detection of liver diseases. AFP is a broadly validated protein for diagnosis of HCC65; however, serum levels of AFP do not allow dis- crimination between HCC and the benign liver diseases. Therefore, an additional tumour marker was proposed, based on a

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glycosylated form of AFP (the AFP-L3 fraction) that shows a highly significant increase in the fucosylation index in HCC patients in comparison to chronic liver diseases196. The fucosylated AFP-L3 fraction was approved by FDA as a marker for early detection of HCC that appears in serum at the stage of liver cirrhosis, just before the onset of HCC, being therefore considered the best approved marker in patients with HCC65,196. Other liver-secreted proteins, such as GP73, kininogen and haptoglobin, have been shown to be fucosylated, acting as promising biomarkers for early detection of HCC and disease progression197. With the advent of new technologies and new methods for glycan analysis, many examples of aberrant glycans associated with cancer were discovered198. The recent application of precise and stable glycogene editing in mammalian cell lines combined with high-throughput mass spectrometry approaches has contributed to the characterization of the O-glycoproteome of cancer cells, disclosing new biological information and generating putative disease biomarkers199,200. In addition, the newly developed high-throughput platform technologies have further enabled the analysis of large cohorts of samples in an efficient manner198,201. An increased concentration of fucosylated haptoglobin occurs in serum of patients with pancreatic cancer compared with that of patients with other types of cancer, such as gastric cancer or CRC, and healthy controls202. Recently, STn antigen was found in circulating CD44 in serum from patients with gastric cancer200. In addition, STn has been found in plasminogen in serum from patients with intestinal metaplasia and gastric carcinoma203. Additional studies showed altered glycosylation (both fucosylation and sialylation) in PSA as a specific biomarker for prostate cancer that is able to distinguish it from benign prostate hyperplasia187,204. Therefore, it is likely that targeting glycans in combination with the protein backbone will provide greater diagnostic and prognostic performance, with sufficient sensitivity and specificity for clinical applications. Additionally, circulating exosomes enriched in certain glycoconjugates have major potential for early detection of cancer. This is the case of proteoglycan glypican 1 (GPC1), which has been shown to identify circulating pancreatic cancer exosomes and allows the early detection of this cancer205. Serum antibodies against tumour-associated glycan antigens have been shown to have potential applications as biomarkers for early cancer detection206. The detection of aberrant glycosylated MUC1-specific autoantibodies correlates with CRC, predicting this cancer with 95% specificity207. However, the low sensitivity of the assay supports the use of it in combination with other markers, suggesting that a combination of antibody signatures may eventually enable a biomarker panel for the early detection of cancer207. Furthermore, microarrays of glyco peptides displaying cancer-associated glycans open new avenues for the expansion of glycoconjugates and glycoforms for further cancer biomarker discovery with potential clinical applications206. In summary, the impressive progress in the understanding the role of glycans in cancer in the recent years has contributed to the discovery of glycans as promising biomarkers, highlighting their application in the clinical setting as appealing targets for personalized medicine180.

Conclusions and perspectives

Glycosylated proteins and other glycoconjugates are major components of cells, defining and modulating several key physiological processes in normal tissues. Genetic, epigenetic, metabolic, inflammatory and environmental mechanisms can lead to modifications of glycosylation that drive several biological processes in cancer. The understanding of the molecular basis underlying these glycan modifications will further contribute to explain cancer cell interactions, extracellular communications (including extracellular vesicles and exosome communication) and cancer immunology. The foreseeable new knowledge in the glycobiology field, with the rapid expansion of

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novel (glyco)engineered cell and model platforms, which are providing increas- ing advances in the understanding of how glycosylation modulates biological functions, will allow the develop- ment of a relatively unexploited field of drugs based on inhibitors, glycan antagonists and glycan-function mod- ulators. Furthermore, the combination of an increasing amount of data on glycomics and glycoproteomics and the recent advances in genomics, transcriptomics, prot- eomics and metabolomics will have a major impact on the unravelling of novel targets and strategies for the early diagnosis, prognosis, patient stratification and improved treatment of cancer.

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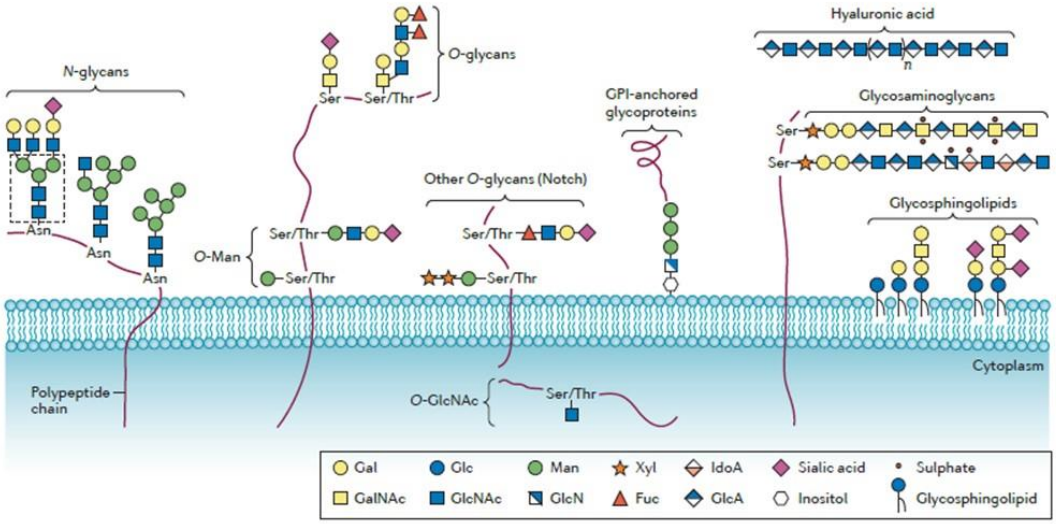

Figure 1 | **Common classes of glycoconjugates in mammalian cells.** Glycans can be found in various types of macromolecules. Glycosphingolipids are major components of the outer leaflet of the cell plasma membrane. These ceramide-linked glycans are made of a variable series of structures that can be further modified with terminal sialic acids8,17. Proteins can be glycosylated by the covalently attachment of a saccharide to a polypeptide backbone, via *N*- linkage to Asp or *O*-linkage to Ser/Thr8. Mucin-type *O*-glycans are frequently found in secreted or membrane-associated glycoproteins and are initiated by *N*-acetylgalactosamine (GalNAc) *O*-linked to Ser/Thr13. *O*-glycans can be extended, producing various 'cores' and different terminal structures that are usually fucosylated and sialylated14. Other types of *O*-glycans include the *O*-mannose (*O*-Man), *O*-fucose (*O*-Fuc), *O*-galactose (*O*-Gal) and nucleocytoplasmic *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc)11,15. *N*-glycosylation occurs in the consensus peptide sequences Asn-X-Ser/Thr (in which X denotes any amino acid). *N*-glycans share a common pentasaccharide core region (highlighted in the figure as a dotted line box) that can be further diversified into oligomannose, hybrid or complex types and further modified by the terminal structures GlcNAc, Gal and sialic acid8. Some glycoproteins can also be found in the outer leaflet of the plasma membrane linked to a phosphatidylinositol; these are called glycosylphosphatidylinositol (GPI)-anchored proteins8. Glycosaminoglycans are linear co-polymers of acidic disaccharide repeating units mostly found attached to the so-called proteoglycans8. An exception is hyaluronic acid, which is a glycosaminoglycan found free in the extracellular matrix.

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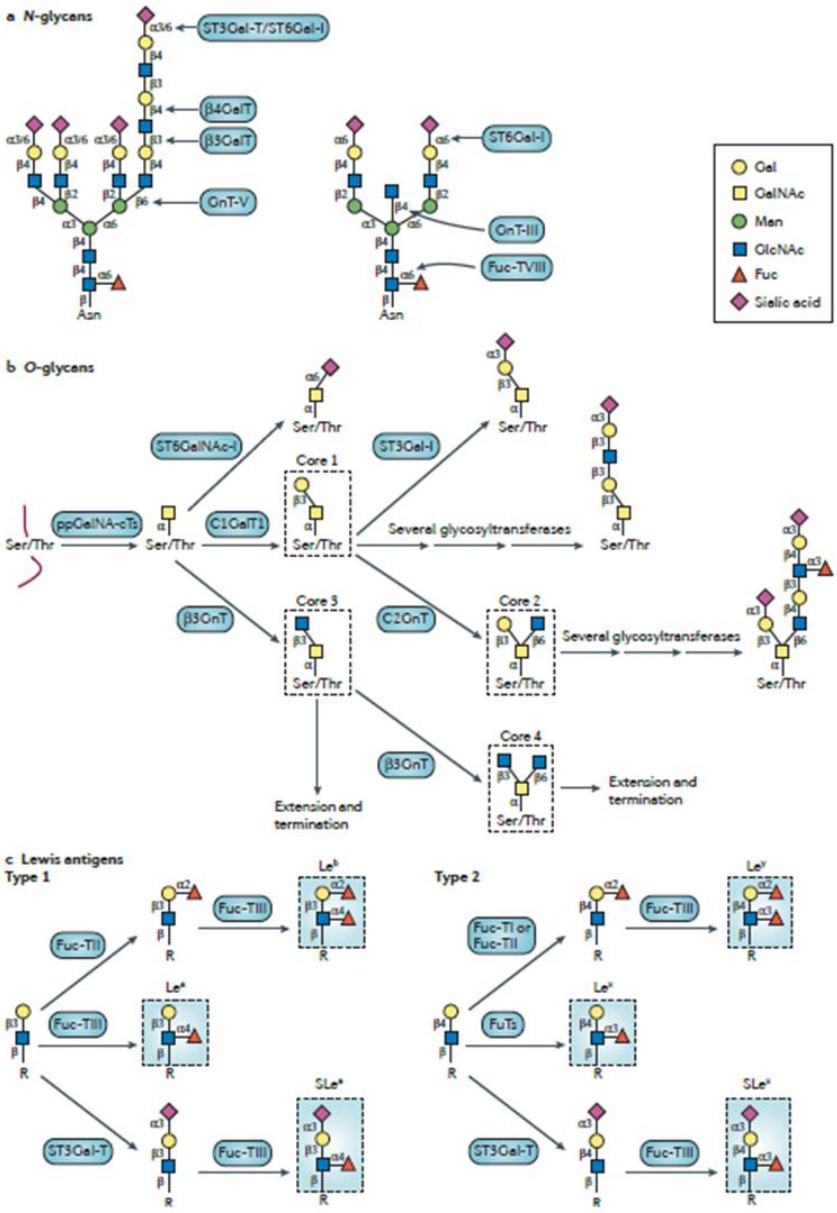

Figure 2 | **Schematic representation of important glycan structures.** The figure represents specific *N*-linked (**a**) and *O*-linked (**b**) glycan structures, as well as the terminal Lewis and sialylated Lewis structures (**c**). The key enzymes responsible for the addition of specific sugar residues are also shown in blue boxes. Examples include the polypeptide *N*-acetylgalactosamine transferases (ppGalNAc-Ts; a family of 20 enzymes, including GalNAc-T1, GalNAc-T2, GalNAc-T3, GalNAc-T4, GalNAc-T5 and GalNAc-T6), sialyltransferases (such as α-galactoside α-2,6-sialyltransferase I (ST6Gal-I), α2,3-sialyltransferases (ST3Gal-Ts) and α-GalNAc ST6Gal-I (ST6GalNAc-I)), *N*-acetylglucosamine (GlcNAc) transferases (GnTs; such as GnT-III, GnT-V, core 2 GnTs (C2GnTs)

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and β3GnT) and fucosyltransferases (Fuc-Ts). The latter include Fuc-TVIII (which mediates the addition of 'core' α1,6Fuc to *N*-glycans); Fuc-TI and Fuc-TII, which add fucose (Fuc) in α1,2 linkage to galactose (Gal); Fuc-Ts that mediate the addition of Fuc in α1,3 linkage to an α2,3-sialylated type 2 chain (Fuc-TIII, Fuc-TIV, Fuc-TV, Fuc-TVI, Fuc-TVII and Fuc-TIX); and Fuc-Ts that add Fuc in α1,4 linkage to an α2,3-sialylated type 1 chain (Fuc-TIII and Fuc-TV). The blue boxes highlighted in part **c** show the carbohydrate terminal Lewis antigens. Lewis type 1 antigens includes Lewis a (Lea), Leb and sialyl Lewis a (SLea); the type 2 group includes Lex, Ley and SLex. C1GalT1, core 1 GalNAc β1,3-GalT 1; GalT, galactosyltransferase; GlcA, glucuronic acid; Man, mannose; STn, sialyl Tn.

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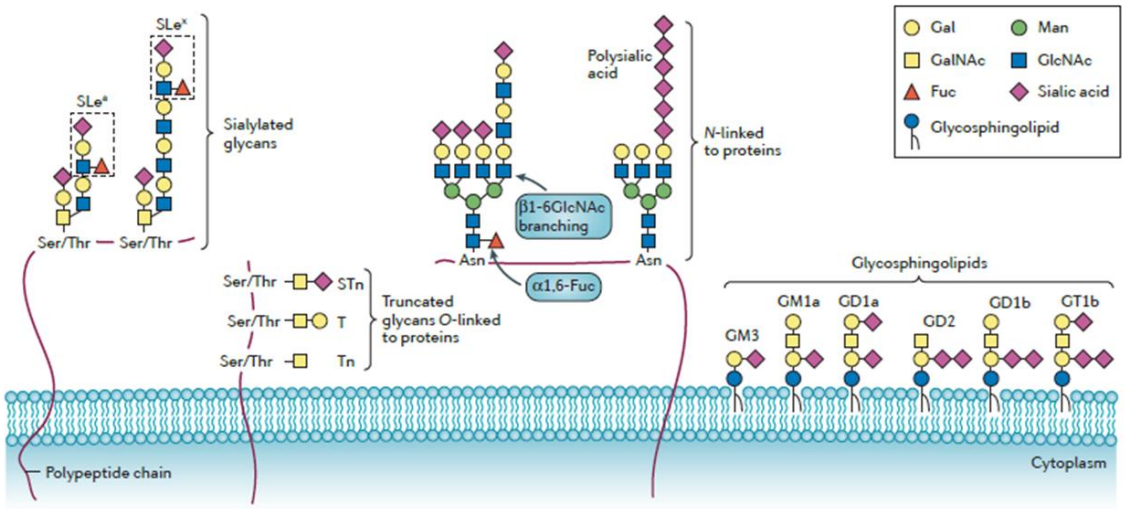

Figure 3 | **Important tumour-associated glycans.** Tumour cells often display glycans with different structures and levels of expression compared with their normal counterparts. These tumour-specific glycans are considered a hallmark of cancer cells. The most-widely occurring changes in glycosylation associated with cancer include an increase in overall sialylation2,25. Aberrant glycosylation in cancer frequently involves an increase in sialyl Lewis x (SLex) and SLea (REF. 41) antigens, as well as an increase in terminal α2,6-sialylated structures, both in truncated *O*-linked glycans (such as sialyl Tn (STn))23,35,38 and in *N*-linked glycans113, and an increase in the α2,8-linked polymer known as polysialic acid52. Moreover, certain sialic acid-containing glycosphingolipids called gangliosides (including monosialogangliosides, such as GM3 and GM1a, disialogangliosides, such as GD1a, GD2 and GD1b, and trisialogangliosides, such as GT1b) have been associated with malignancy17. Another broadly occurring change in glycosylation associated with cancer is an enhancement of β1,6-*N*-acetylglucosamine (β1,6GlcNAc)-branched structures in *N*-linked glycans caused by an increased activity of *N*-acetylglucosaminyltransferase V (GnT-V)67. Overexpression of 'core' fucosylation (the addition of α1,6-fucose (α1,6-Fuc) to the innermost GlcNAc of *N*-glycans) by fucosyltransferase VIII (Fuc-TVIII) is also considered an important event in tumour development and progression196.Gal, galactose; GalNAc, *N*-acetylgalactosamine; Man, mannose.

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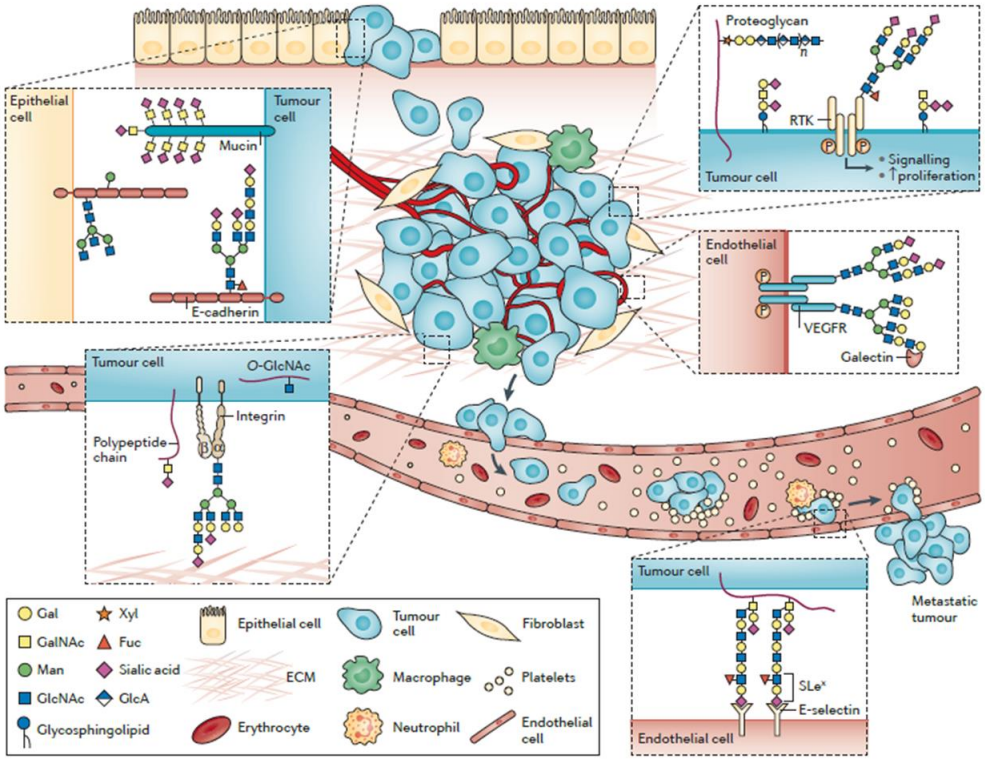

Figure 4 | **Role of glycans in cancer development and progression.** Glycans play fundamental parts in key pathological steps of tumour development and progression. In the process of tumour cell dissociation and invasion, glycans interfere with cell–cell adhesion. The modification of epithelial cadherin (E-cadherin) with β1,6-*N*-acetylglucosamine (β1,6GlcNAc)-branched *N*-glycan structures through enhanced *N*-acetylglucosaminyltransferase V (GnT-V) activity impairs cell adhesion and promotes tumour cell invasion94. These branched structures can be extended and the α2,6-sialylated terminal structures interfere with tumour cell adhesion. The presence of E-cadherin *N*-glycans with bisecting GlcNAc structures catalysed by GnT-III leads to protein stability and suppression of tumour progression75,94,95. Aberrant *O*-glycosylation, such as expression of sialyl Tn (STn) owing to overexpression of α-*N*-acetylgalactosamine (α-GalNAc) α-2,6-sialyltransferase I (ST6GalNAc-I)36,38 or mutations in C1GalT1-specific chaperone 1 (*C1GALT1C1*), is also associated with tumour cell invasion84,85. The process of tumour growth and proliferation is characterized by altered glycosylation of key growth factors receptors, which modulates their activity and signalling161. Expression of gangliosides in the cancer cell membrane can also modulate signal transduction, activating various cellular pathways that induce tumour growth and progression17. Altered *O*-GlcNAcylation is also associated with cancer progression153. In the process of tumour cell migration, integrins show altered glycosylation in both *O*-linked and *N*-linked glycans76. Terminal sialylation interferes with cell–extracellular matrix (ECM) interactions, promoting an increased migratory and invasive phenotype140. The aberrant glycosylation of vascular endothelial growth factor receptor (VEGFR) modulates its interaction with galectins and is associated with

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tumour angiogenesis69. The tumour-associated carbohydrate determinants sialyl Lewis x (SLex) and SLea serve as ligands for the adhesion receptors expressed in activated endothelial cells (E-selectin), platelets (P-selectin) and leukocytes (L-selectin), promoting cancer cell adhesion and metastasis46. Fuc, fucose; Gal, galactose; GlcA, glucuronic acid; Man, mannose; RTK, receptor tyrosine kinase; Xyl, xylose.

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Serological marker	Glycoprotein or glycoform	Cancers	Application in the clinic	Refs
AFP	AFP 'core' fucosylation (AFP-L3)	Hepatocellular carcinoma	Early diagnosis and monitoring	65,196
CA19-9	SLe^a	Biliary, colorectal, gastric and pancreatic	Therapeutic monitoring, recurrence and tumour burden	49,192
CA72-4	STn	Gastric	Monitoring	219
CA15-3	MUC1	Breast	Monitoring	190,191
CA125	MUC16	Ovarian	Monitoring and recurrence	188
CEA	CEA	Colorectal	Monitoring and recurrence	189
PSA	PSA	Prostate	Diagnosis, monitoring and recurrence	187
β-hCG	β-hCG	Gynaecological	Monitoring	220

β-hCG, β-human chorionic gonadotropin; AFP, α-fetoprotein; CA, cancer antigen; CEA, carcinoembryonic antigen; MUC, mucin; PSA, prostate specific antigen; SLe^a, sialyl Lewis a; STn, sialyl Tn.

Table 1 | Examples of serological markers with clinical applications

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